Genetic and environmental influences on the yields of pentane extractable hydrocarbons of *Helianthus annuus* (Asteraceae, Sunflowers)

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ABSTRACT

Sunflowers (*H. annuus*) from natural populations in the Texas Panhandle and Salt Lake City, UT were analyzed for their % HC yields and g HC/g DW 10 leaves. Seeds from these populations were germinated and plants grown in the OPSU greenhouse to investigate the influence of the environment on HC yields. The ratio of % HC yields for greenhouse (GH) plants vs natural (Nat) plants was (GH/Nat): GT: 45.9%; LT: 55.6%; SLC: 78.3%). Plants from both of the populations in the Texas Panhandle (GT, Gruver, TX; LT, Lake Tanglewood, TX) had yields about 50% higher than their progeny grown under greenhouse conditions. Plants from these natural populations were severely damaged by grasshoppers, other insects and pathogens, as well as high winds and large variations in rainfall during the season (water stress). In contrast, the SLC (Salt Lake City, UT) population was an urban population that appeared to have much less insect damage to the leaves and no significant wind damage. Yields of HC (g/g leaf basis) were also much lower in greenhouse plants, but this is largely due to the lower biomass produced in small pots. Hybrids obtained by crossing two high yielding native sunflowers were greenhouse grown and these produced much less HC yields (4.59%, P1xP2, 4.91%, P2xP1) than found in their high yielding, natural habitat, parents (9.44 P1, 10.03 P2 %). These experiments indicate that HC yields from sunflower leaves are greatly induced (up to 55%) by stresses in the environment. Published on-line www.phytologia.org *Phytologia 99(2):186-190 (May 9, 2017).* ISSN 030319430.

KEY WORDS: *Helianthus annuus,* Sunflower, greenhouse grown, field grown, stress induced hydrocarbon synthesis.

Recently, Adams et al. (2017) reported on geographic variation in pentane extractable hydrocarbons (HC) in natural populations of *Helianthus annuus* L. Yields of HC were highest in the Gruver, TX (GT, 7.99%) and Lake Tanglewood, TX (LT, 7.89%) populations. The leaves of these populations were severely damaged by grasshoppers, other insects and pathogens. It may be that insect damage (and wind damage, etc.) induced synthesis of defense chemicals that led to these high amounts of HC yields.

To test this effect, seeds were collected from these populations plus the Salt Lake City (SLC) population and germinated. The plants were then grown in a greenhouse and leaves harvested at stage R 5.1 - R 5.3 (as done in the field sampling study). The purpose of this paper is to present a comparison of the HC yields from natural and greenhouse (protected and sheltered) plants of *H. annuus* to examine the effects on HC yields when growing under quite different environmental conditions.

This is a part of a continuing study on the development of sunflowers as a source for natural rubber and bio-fuels from the biomass (Adams, et al., 1986; Adams and Seiler, 1984; Pearson et al., 2010a,b; Seiler, Carr and Bagby, 1991).

MATERIALS AND METHODS

Population locations for seed collections:
Lake Tanglewood, TX (LT) - Field notes:
2- 3 ft plants, lots of resin on petioles and leaf veins, many sugar ants (black ants), most with wilted leaves, very dry in July, common in native grass and on disturbed roadside, brush dump area, Lake Tanglewood, ~50% flowering, 35° 04' 23.7" N, 101° 47' 29.0" W, 3239 ft., Date: 12 July 2016, County: Randall; State: TX Coll. Robert P. Adams No. 14947

Gruver, TX (GT) - Field notes:
2-3' tall, 10% flowering, lots of damage to leaves by grasshoppers, etc., some with many sugar ants, copious resin at base of leaves, along fence row, on TX 206, 1-5:1.2 mi s, 6-10: 1.3 mi. s of Gruver, TX. 36° 14' 52" N, 101° 24' 52" W, 3161 ft, Date: 16 July 2016, County: Hansford; State: TX
Coll. Robert P. Adams No. 14952

Salt Lake City, UT (SLC) - Field notes:
next to sidewalk, flowering and seeding, multiple branches. common along sidewalks, Mill Creek, UT. s side of I80 on 2000 E, east side of 2000 E. 42° 52' 49" N, 112°' 25' 35" W, 4625 ft, Date: Sept. 3, 2016, County: Salt Lake; State: Utah. Coll. Robert P. Adams No. 15026

Bulk seed collections were made from LT, GT and SLC populations and the seeds stratified at 4°C for 1 week in a 1000 ppm GA3 solution (Adams and TeBeest, 2016, 2017), then placed on moist filter paper inside petri dishes. Seeds germinated in 10 - 14 days and seedlings were transplanted to potting soil in seed tube flats. After two weeks, the seedlings were transplanted into 7" round pots. The plants were grown the OPSU greenhouse were watering as needed with 12 hrs. supplemental light.

The lowest growing, non-yellowed, 8 mature leaves were collected at stage R 5.1-5.3 when the first flower head opened with mature rays when the HC yields are reported as highest for sunflowers (Adams et al. 2016). The leaves were air dried in paper bags at 49° C in a plant dryer for 24 hr or until 7% moisture was attained.

Leaves were ground in a coffee mill (1mm). 3 g of air dried material (7% moisture) were placed in a 125 ml, screw cap jar with 20 ml pentane, the jar sealed, then placed on an orbital shaker for 18 hr. The pentane soluble extract was filtered through a Whatman paper filter into a pre-weighed aluminum pan and the pentane evaporated on a hot plate (50°C) in a hood. The pre-weighed aluminum pan with concentrated hydrocarbon extract was weighed and tared.

RESULTS

Table 1 shows the biomass (DW, 10 leaves), % HC yields, and g HC/gDW 10 leaves for sunflowers growing at Gruver, Lake Tanglewood, and Salt Lake City from natural populations and for plants grown in the OPSU greenhouse. In general, the plants were much more robust in the field and the yields were much higher in nature than in the greenhouse.

These trends are shown graphically in Figure 1. The DW of 10 leaves from plants grown in the greenhouse was only 13% to 23% as much as field grown plants. Because the greenhouse plants were grown in 7" pots, this limited their growth. In addition, the greenhouse had a 50% shade cloth that limited the sunlight. Greenhouse sunflowers had elongated stems compared to natural field sunflowers.

Table 1. Yields of hydrocarbons (HC) *H. annuus*, from three natural populations and progeny obtained by growing field collected, bulk seed, then germinating and growing these progeny in the greenhouse. 2 x Standard error the mean computed as 2 x standard deviation / $(n)^{1/2}$. Xm = mean, 2Se = 2 x standard error of mean (95.6% of samples).

	naturally grown Xm±2SE			greenhouse grown Xm±2SE		
population sampled	g DW 10 lvs	% yield	g/ DW 10 lvs	g DW 10 lvs	% yield	g/ DW 10 lvs
14952 Gruver, TX	19.36 ± 2.010(40)	7.99± 0.537	1.499± 0.137	2.54± 0.178(60)	3.67± 0.249	0.092± 0.00798
14947 Lake Tanglewood, TX	19.11 ± 1.997(20)	7.88 ± 0.735	1.484 ± 0.200	2.79± 0.446(14)	4.38± 0.858	0.120± 0.0265
15026 Mill Creek, Salt Lake City, UT	11.92 ± 3.883(20)	5.75± 0.968	0.672± 0.195	2.69± 0.366(17)	4.50± 0.436	0.121± 00213

The ratio of % HC yields for greenhouse (GH) plants vs natural (Nat) plants was (GH/Nat): GT: 45.9%; LT: 55.6%; SLC: 78.3%, Fig. 1, Table 1). Progeny from both of the Texas Panhandle (GT, Gruver, TX; LT, Lake Tanglewood, TX) populations had yields about 50% lower when grown under greenhouse conditions. Plants from these populations were severely damaged by grasshoppers, other insects and pathogens as well as high winds and large variations in rainfall during the season (water stress). In contrast, the SLC (Salt Lake City, UT) population was an urban population that appeared to have much less insect damage to the leaves and no significant wind damage. Of course, it was subject to fluctuations in moisture.

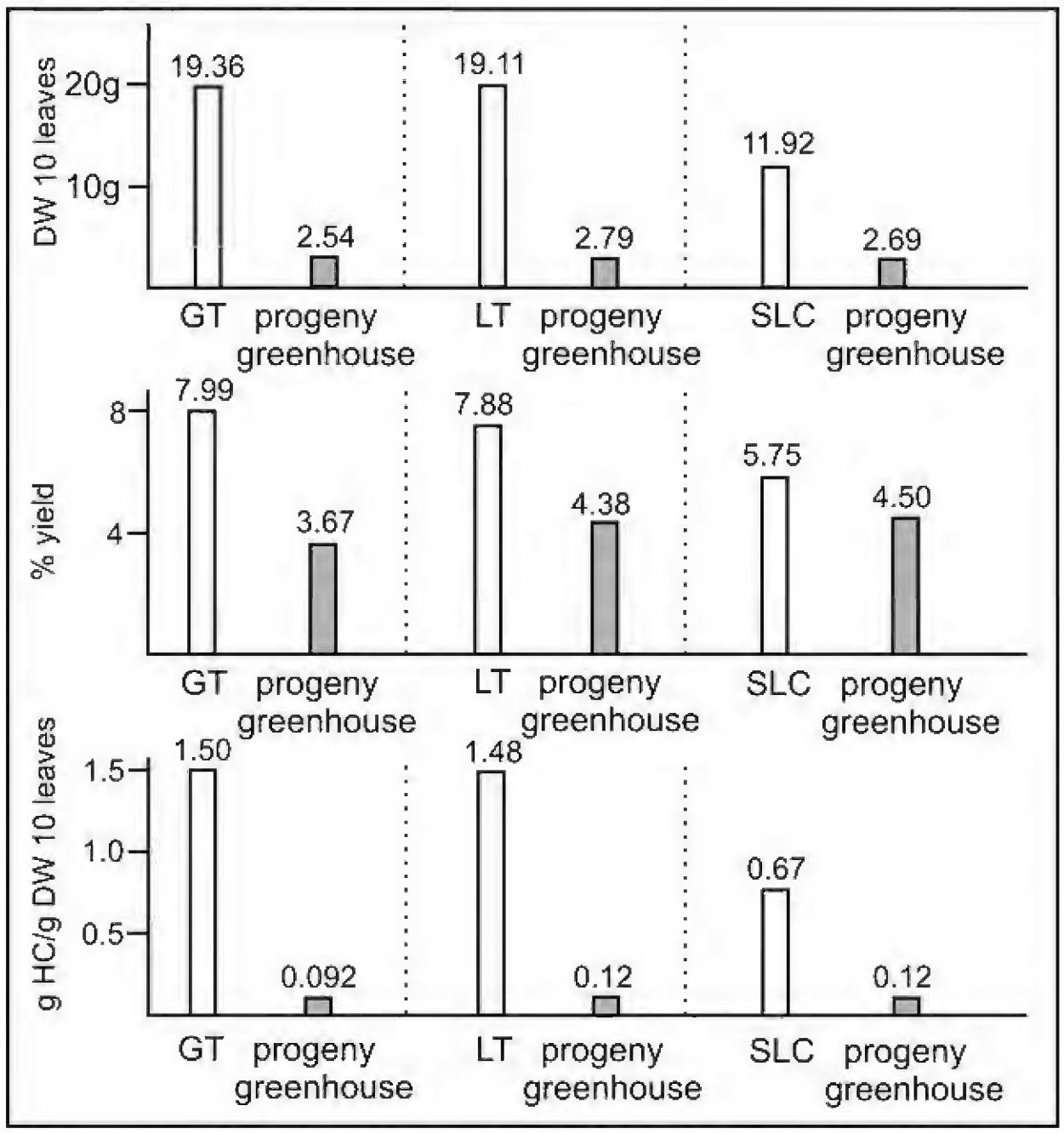

Figure 1. Comparisons of DW 10 leaves, % HC yield, and g HC/ g DW 10 leaves for field sampled sunflowers vs their progeny grown in the greenhouse at OPSU.

Hybrids produced by crossing two high yielding, native sunflowers (GT29, GT30, Gruver, TX), were grown in the OPSU greenhouse. These hybrid plants had much smaller yields (4.59%, 4.91% Table 2) than found in their high yielding, naturally grown parents (9.44, 10.03%, Table 2.). In addition, the hybrids (from the cross and the reciprocal cross) also had much lower HC concentrations (g HC/ g DW 10 lvs, Table 2).

Table 2. Production of HC from progeny of crossing high yielding individuals P1, GT 29 (9.44% HC, 1.417 g HC/ DW 10 leaves) and P2, GT 30 (10.03%, 0.917 g HC/ 10 leaves). Cross I = P1, GT29(male) x P2, GT30(female), Cross II = P1, GT29(female) x P2, GT30 (male). GT29 and GT30 were naturally growing *H. annuus* transplanted from the field in Gruver, TX, to the greenhouse. Crosses were made under controlled conditions in the greenhouse. Selfing in GT29 and in GT30 resulted in no seeds showing that these *H. annuus* are self-sterile. Progeny were grown in the greenhouse.

crosses	g DW 10 lvs	% yield	g/ DW 10 lvs
Parent 1, GT29	15.01g	9.44%	1.42g
Parent 2. GT30	9.14g	10.03%	0.92g
Cross I = P1, GT29(male) x P2, GT30(female) 26 hybrids	2.42g± 0.156	4.81%± 0.506	0.116g± 0.0149
Cross II = P1, GT29 (female) x P2 GT30 (male) 25 hybrids	2.97g± 0.257	4.59%± 0.450	0.139g± 0.020

The pattern of plants from the natural environment (with insect, pathogens and wind damage as well as moisture stress) having higher HC yields than plants from the protected greenhouse environment is shown in Figure 2. These data are consistent with the results previously shown (Table 1, Fig. 1) for field population samples vs. greenhouse grown plants from bulk seed collections.

Thus, it appears, in these cases, that up to 55% of the HC concentrations in natural populations of *H. annuus* in the Texas Panhandle are induced by the environment. The SLC (Salt Lake City) population was under less stress, but even that stress resulted in 22% greater HC concentration than in the non-stressed greenhouse plants.

Although these results are preliminary, they do show that growing sunflowers in a lush, sheltered environment (i.e., greenhouse) is not efficient for the production of high yields of HC. These results support the idea that much of the HC synthesis in sunflowers (up to 55%) is induced by stress to the plants. How much of that synthesis is due to insect and/ or pathogen damage vs. moisture stress is not known.

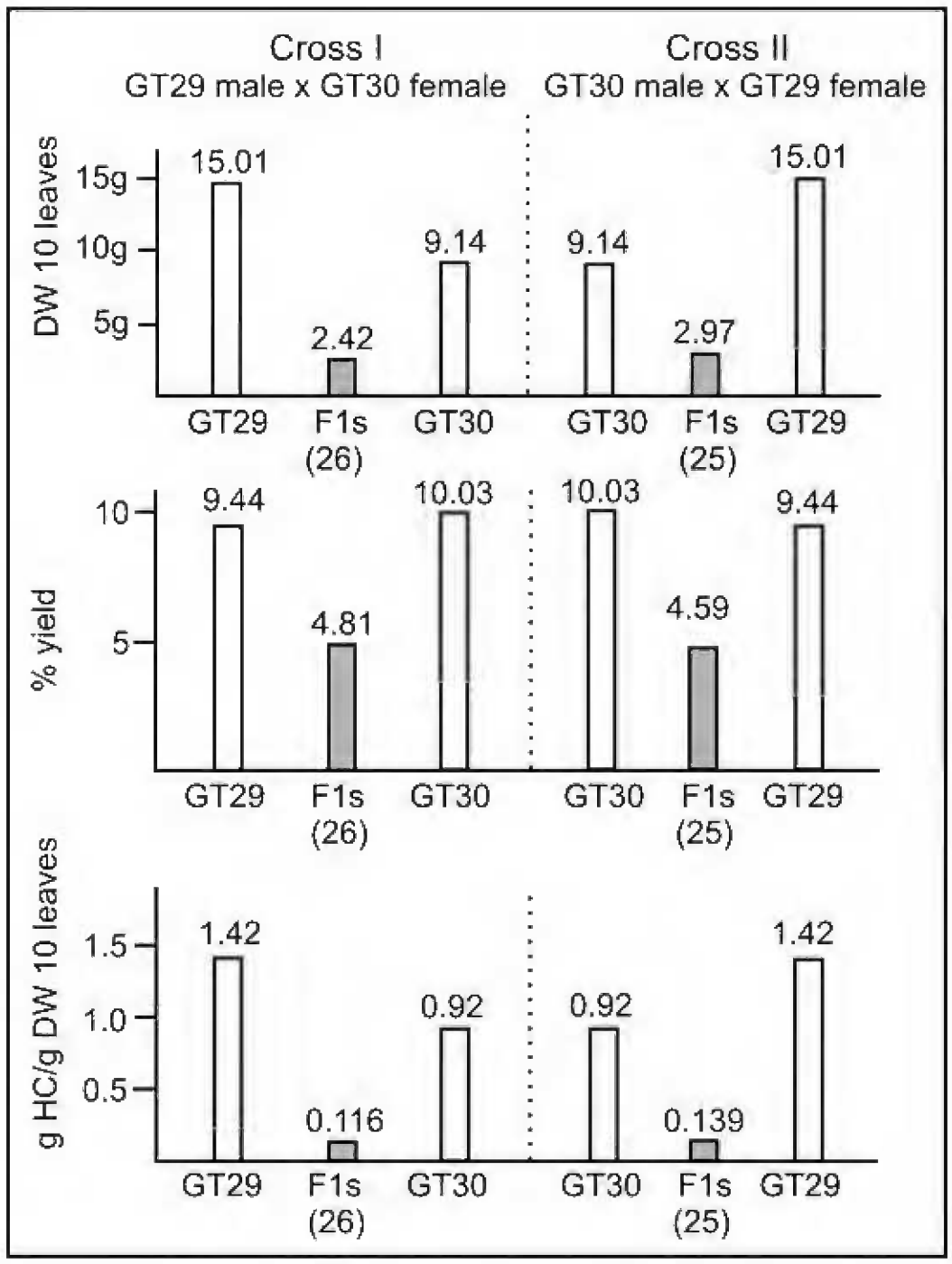

Figure 2. Comparison of DW 10 leaves, % HC yield, and g HC/g DW 10 leaves for field grown sunflowers (P1, GT29, P2, GT30) vs hybrids grown in the greenhouse at OPSU.

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